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Native and solubilized decellularized extracellular matrix: a critical assessment of their potential for improving the expansion of mesenchymal stem cells.

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Abstract

Capturing the promise of mesenchymal stem cell (MSC)-based treatments is currently limited by inefficient production of cells needed for clinical therapies. During conventional ex vivo expansion, a large portion of MSCs lose the properties that make them attractive for use in cell therapies. Decellularized extracellular matrix (dECM) has recently emerged as a promising substrate for the improved expansion of MSCs. MSCs cultured on these surfaces exhibit improved proliferation capacity, maintenance of phenotype, and increased differentiation potential. Additionally, these dECMs can be solubilized and used to coat new cell culture surfaces, imparting key biological properties of the native matrices to other surfaces such as tissue engineering scaffolds. Although this technology is still developing, there is potential for an impact in the fields of MSC biology, biomaterials, tissue engineering, and therapeutics. In this article, we review the role of dECM in MSC expansion by first detailing the decellularization methods that have been used to produce the dECM substrates; discussing the shortcomings of current decellularization methods; describing the improved MSC characteristics obtained when the cells are cultured on these surfaces; and considering the effect of the passage number, age of donor, and dECM preparation method on the quality of the dECM. Finally we describe the critical roadblocks that must be addressed before this technology can fulfil its potential, including elucidating the mechanism by which the dECMs improve the expansion of primary MSCs and the identification of a readily available source of dECM.

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Characterisation and intracellular labelling of mesenchymal stromal cells derived from synovial fluid of horses and sheep.

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Abstract

Multipotent mesenchymal stromal cells (MSCs) derived from synovial fluid (SF) are considered to be a promising cell type for therapeutic applications in joint disease. However, despite their potential relevance for clinical and experimental studies, there is insufficient knowledge about SF-derived MSCs isolated from horses and sheep. In this study, cells were recovered from healthy SF and bone marrow

(BM) of sheep, and from healthy and osteoarthritic SF of horses. Ovine SF-MSCs were used to assess the efficiency of intracellular labelling with quantum dots (QDs). Colony forming units, generation times, trilineage differentiation potential and expression of CD73, CD90 and CD105 at mRNA level were assessed. QD labelling was efficient, with >98% positive cells directly after labelling at 10 nmol/L and >95% positive cells directly after labelling at 2 nmol/L. The label decreased over 7 days of culture, with more persistence at the higher labelling concentration. No significant differences in proliferation were observed. All MSCs had trilineage differentiation potential, but adipogenesis was more distinct in equine samples and chondrogenesis was most pronounced in ovine SF-MSCs. CD73, CD90 and CD105 were expressed in equine and ovine MSCs.

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Surface Atomic Structure Directs the Fate of Human Mesenchymal Stem Cells.

Dong L, Cheng K, Zhou Y, Yu M, Gong J, Lin Y, Luo Q, Wang Q, Weng WJ, Wang H.

Abstract

Stem cells in contact with materials are able to sense their surface features, integrate extracellular matrix (ECM) protein cues via signal transduction pathway and ultimately direct cell fate decisions. However, discovering the interdisciplinary mechanisms of how stem cells response to inherent material surface features still remains a challenge due to the complex, multicomponent signalling milieu present in ECM environment. Here, we demonstrate that the fate of human mesenchymal stem cells (hMSCs) can be regulated by inherent physical cue of material surface down to atomic-scale features. hMSCs on TiO-terminated SrTiO3 {110} substrate tend to differentiate into specific lineage cells (osteoblast, chondrocyte, adipocyte) while they are prone to maintain pluripotency on TiO2-terminated SrTiO3 {100} substrate. The experimental observations and molecular dynamics (MD) simulations indicate that the distinct conformations of initial adsorbed serum albumin and fibronectin proteins activate the integrin-focal adhesion cytoskeleton actin transduction pathway and subsequently direct the gene and protein expressions of hMSCs. Moreover, we demonstrate that the initial protein adsorption behaviors are dependent on the distinct hydroxyl groups originate from different surface atomic structures as well as the work functions. This work therefore provides new insights into the fundamental understanding of cell-material interactions, and will have a profound impact on further designing materials to direct stem cell fate.

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Spheroid Coculture of Hematopoietic Stem/Progenitor Cells and Monolayer Expanded Mesenchymal Stem/Stromal Cells in Polydimethylsiloxane Microwells Modestly Improves In Vitro Hematopoietic Stem/Progenitor Cell Expansion.

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Abstract

While two-dimensional (2D) monolayers of mesenchymal stem/stromal cells (MSCs) have been shown to enhance hematopoietic stem/progenitor cell (HSPC) expansion in vitro, expanded cells do not engraft long term in human recipients. This outcome is attributed to the failure of 2D culture to recapitulate the bone marrow (BM) niche signal milieu. Herein, we evaluated the capacity of a novel three-dimensional (3D) coculture system to support HSPC expansion in vitro. A high-throughput polydimethylsiloxane (PDMS) microwell platform was used to manufacture thousands of uniform 3D multicellular coculture spheroids. Relative gene expression in 3D spheroid versus 2D adherent BM-derived MSC cultures was characterized and compared with literature reports. We evaluated coculture spheroids, each containing 25-400 MSCs and 10 umbilical cord blood (CB)-derived $CD34^+$ progenitor cells. At low exogenous cytokine concentrations, 2D and 3D MSC coculture modestly improved overall hematopoietic cell and $CD34^+$ cell expansion outcomes. By contrast, a substantial increase in $CD34^+CD38^-$ cell yield was observed in PDMS microwell cultures, regardless of the presence or absence of MSCs. This outcome indicated that $CD34^+CD38^-$ cell culture yield could be increased using the microwell platform alone, even without MSC coculture support. We found that the increase in $CD34^+CD38^-$ cell yield observed in PDMS microwell cultures did not translate to enhanced engraftment in NOD/SCID gamma (NSG) mice or a modification in the relative human hematopoietic lineages established in engrafted mice. In summary, there was no statistical difference in $CD34^+$ cell yield from 2D or 3D cocultures, and MSC coculture support provided only modest benefit in either geometry. While the high-throughput 3D microwell platform may provide a useful model system for studying cells in coculture, further optimization will be required to generate HSPC yields suitable for use in clinical applications.

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Gelatin-derived graphene-silicate hybrid materials are biocompatible and synergistically promote BMP9-induced osteogenic differentiation of mesenchymal stem cells (MSCs).

Zou Y, Taheri Qazvini N, Zane K, Sadati MS, Wei Q, Liao J, Fan J, Song D, Liu J, Ma C, Qu X, Chen L, Yu X, Zhang Z, Zhao C, Zeng Z, Zhang R, Yan S, Wu T, Wu X, Shu Y, Li Y, Zhang W, Reid RR, Lee MJ, Wolf JM, Tirrell M, He TC, de Pablo JJ, Deng ZL.

Abstract

Graphene-based materials have been used in many fields but have only found limited applications in biomedicine, including bone tissue engineering. Here we demonstrate that novel hybrid materials consisting of gelatin-derived graphene and silicate nanosheets of Laponite (GL) are biocompatible and promote osteogenic differentiation of mesenchymal stem cells (MSCs). Homogeneous cell attachment, long-term proliferation and osteogenic differentiation of MSCs on GL-scaffold were confirmed by optical microscopy, and scanning electron microscopy. GL-powders made by pulverizing GL-scaffold were shown to promote BMP9-induced osteogenic differentiation. GL-powders increased ALP activity in

iMEFs, but decreased ALP activity in more differentiated iMADs. Note, however, that GL-powders promoted BMP9-induced calcium mineral deposits in both MSC lines, as assessed by qualitative and quantitative Alizarin Red assay. Furthermore, expression of chondro-osteogenic regulator markers such as Runx2, Sox9, osteopontin and osteocalcin was up-regulated by the GL-powder independent of BMP9 stimulation, although the powder synergistically up-regulated BMP9-induced Osterix expression, the adipogenic marker PPARγ was unaffected. Furthermore, in vivo stem cell implantation experiments demonstrated that GL-powder could significantly enhance the BMP9-induced ectopic bone formation from MSCs. Collectively, our results strongly suggest that the GL hybrid materials promotes BMP9-induced osteogenic differentiation of MSCs, and holds promise for the development of bone tissue engineering platforms.

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An engineered multiphase 3D microenvironment to ensure the controlled delivery of cyclic strain and hGDF-5 for the tenogenic commitment of hBMSCs.

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Abstract

At present, injuries or rupture of tendons are treated by surgical repair or conservative approaches with unpredictable clinical outcome. Alternative strategies to repair tendon defects without the undesirable side effects associated with the current options are needed. With this in mind, a tissue engineering approach has gained considerable attention as a promising strategy. Here, we investigated a synthetic 3D microenvironment able to interact with stem cells and inducing, via coupled biochemical and physical signals, their early commitment towards the tenogenic lineage. This multiphase 3D construct consisted of a braided hyaluronate elastic band merged with human Bone marrow Mesenchymal Stem Cells (hBMSCs) and poly-lactic-co-glycolic acid microcarriers loaded with human growth differentiation factor 5 (hGDF-5) by means of fibrin hydrogel. The multiphase structure allowed hBMSCs culture under cyclic strain within a microenvironment where a controlled amount of hGDF-5 was regularly delivered. The cooperative biochemical and physical stimuli induced significantly increased expression of tenogenic markers, such as collagen type I and III, decorin, scleraxis and tenascin-C, within only three days of dynamic hBMSCs culture. This approach opens exciting perspectives for future development of engineered tendon tissue substitutes.

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Gene expression profiling of bone marrow mesenchymal stem cells from Osteogenesis Imperfecta patients during osteoblast differentiation.

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Abstract

Mesenchymal stem cells (MSCs) are precursors present in adult bone marrow that are able to differentiate into osteoblasts, adipocytes and chondroblasts that have gained great importance as a source for cell therapy. Recently, a number of studies involving the analysis of gene expression of undifferentiated MSCs and of MSCs in the differentiation into multiple lineage processes were observed but there is no information concerning the gene expression of MSCs from Osteogenesis Imperfecta (OI) patients. Osteogenesis Imperfecta is characterized as a genetic disorder in which a generalized osteopenia leads to excessive bone fragility and severe bone deformities. The aim of this study was to analyze gene expression profile during osteogenic differentiation from BMMSCs (Bone Marrow Mesenchymal Stem Cells) obtained from patients with Osteogenesis Imperfecta and from control subjects. Bone marrow samples were collected from three normal subjects and five patients with OI. Mononuclear cells were isolated for obtaining mesenchymal cells that had been expanded until osteogenic differentiation was induced. RNA was harvested at seven time points during the osteogenic differentiation period (D0, D+1, D+2, D+7, D+12, D+17 and D+21). Gene expression analysis was performed by the microarray technique and identified several differentially expressed genes. Some important genes for osteoblast differentiation had lower expression in OI patients, suggesting a smaller commitment of these patient's MSCs with the osteogenic lineage. Other genes also had their differential expression confirmed by RT-qPCR. An increase in the expression of genes related to adipocytes was observed, suggesting an increase of adipogenic differentiation at the expense osteogenic differentiation.

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Perfusion bioreactor-based cryopreservation of 3D human mesenchymal stromal cell tissue grafts.

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Abstract

The generation of an off-the-shelf in vitro engineered living tissue graft will likely require cryopreservation. However, the efficient addition and removal of cryoprotective agents (CPA) to cells throughout the volume of a three-dimensional (3D) tissue graft remains a significant challenge. In this work, we assessed whether a perfusion bioreactor-based method could be used to improve the viability of cryopreserved mesenchymal stromal cell- (MSC) based tissue constructs as compared to using conventional diffusion-based methods. The bioreactor was first used to saturate 3D constructs with CPA under perfused flow. Following cryopreservation, the bioreactor was then also used for the efficient removal of the CPA from the 3D tissues. We demonstrate that addition and removal of CPA under perfused flow significantly increased the viability of MSC within cryopreserved 3D tissue constructs as compared to conventional diffusion-based methods

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Efficacy of stem cells on periodontal regeneration: Systematic review of pre-clinical studies.

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Abstract

This systematic review aims to evaluate mesenchymal stem cells (MSC) periodontal regenerative potential in animal models. MEDLINE, EMBASE and LILACS databases were searched for quantitative pre-clinical controlled animal model studies that evaluated the effect of local administration of MSC on periodontal regeneration. The systematic review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement guidelines. Twenty-two studies met the inclusion criteria. Periodontal defects were surgically created in all studies. In seven studies, periodontal inflammation was experimentally induced following surgical defect creation. Differences in defect morphology were identified among the studies. Autogenous, alogenous and xenogenous MSC were used to promote periodontal regeneration. These included bone marrow-derived MSC, periodontal ligament (PDL)-derived MSC, dental pulp-derived MSC, gingival margin-derived MSC, foreskin-derived induced pluripotent stem cells, adipose tissue-derived MSC, cementum-derived MSC, periapical follicular MSC and alveolar periosteal cells. Meta-analysis was not possible due to heterogeneities in study designs. In most of the studies, local MSC implantation was not associated with adverse effects. The use of bone marrow-derived MSC for periodontal regeneration yielded conflicting results. In contrast, PDL-MSC consistently promoted increased PDL and cementum regeneration. Finally, the adjunct use of MSC improved the regenerative outcomes of periodontal defects treated with membranes or bone substitutes. Despite the quality level of the existing evidence, the current data indicate that the use of MSC may provide beneficial effects on periodontal regeneration. The various degrees of success of MSC in periodontal regeneration are likely to be related to the use of heterogeneous cells. Thus, future studies need to identify phenotypic profiles of highly regenerative MSC populations.